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ARTERIAL HOMOGRAFTS

I. The Fate of Preserved Aortic Grafts in the Dog

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THE fact that fresh and preserved arterial homografts properly transplanted may be expected to function indefinitely was demonstrated experimentally over 40 years ago by Carrel and Guthrie. Largely because of technical pitfalls, however, arterial homografts in the human have never been extensively used. The need for an effective method for bridging a gap in major blood vessels was brought into sharp focus during the recent war, and current advances in the field of vascular surgery have continued to stimulate research on this problem. It has become increasingly apparent that vein-lined tubes and plastic or metal conduits are not a satisfactory solution, and since the war renewed interest has been aroused in the potentialities of the arterial homograft.

It was our purpose in this study to investigate by histological methods the fate of stored aortic homografts in the dog, and to evaluate the effect of the duration of storage upon the biological and functional properties of the transplanted vessel.

METHOD OF STORAGE OF GRAFTS

If a blood vessel "bank" is to be practical for widespread use in either civilian or military hospitals, a method of storage must be devised which is simple and requires little or no specialized or complicated equipment or solutions. For this reason, we began by using as a storage medium commercially obtained sterile Ringer's solution to which was added 10 per cent by volume freshly prepared sterile dog serum. This solution proved to be quite successful, and grafts so stored would remain in an excellent state of preservation for periods up to 6 months. At first penicillin and streptomycin were added, but in the later phases of our work we found this to be unnecessary if careful sterile precautions were observed in the preparation of the solution. We had no instance of known bacterial contamination occur in our laboratory.

The entire descending aorta from just beyond the arch to just above the bifurcation was removed under sterile precautions within 1 to 4 hours after death of the donor animal. Each efferent artery was carefully ligated by a simple tie of No. 000 silk as close to the wall of the aorta as possible. The entire artery was then placed in a sterile Mason fruit jar containing 450 cubic centimeters of the storage solution, which was tightly stoppered, and kept in a common electric refrigerator at a temperature of 3 to 8 degrees C. By observing care, this vessel could be used serially for several transplants replacing the vessel into the original bottle between implantations.

Pierce and his co-workers have recently reported their careful studies on various methods for the storage of vascular segments. They concluded that the use of a balanced salt solution containing 10 per cent plasma in stoppered bottles maintained at a temperature of 6 degrees to 11 degrees was the most satisfactory method studied. We believe, however, that the carefully evolved but complex formula for buffered salt solution recommended by Pierce is not necessary, and that commercial Ringer's solution is quite adequate.

Our method, which proved to be eminently satisfactory, requires no agents or materials not available in any civilian or military hospital, up to and including the field hospital.

FACTORS OF IMPORTANCE IN THE SUCCESSFUL TRANSPLANTATION OF STORED HOMOGRAFTS

Gross and his co-workers have stressed three factors which might influence the success or

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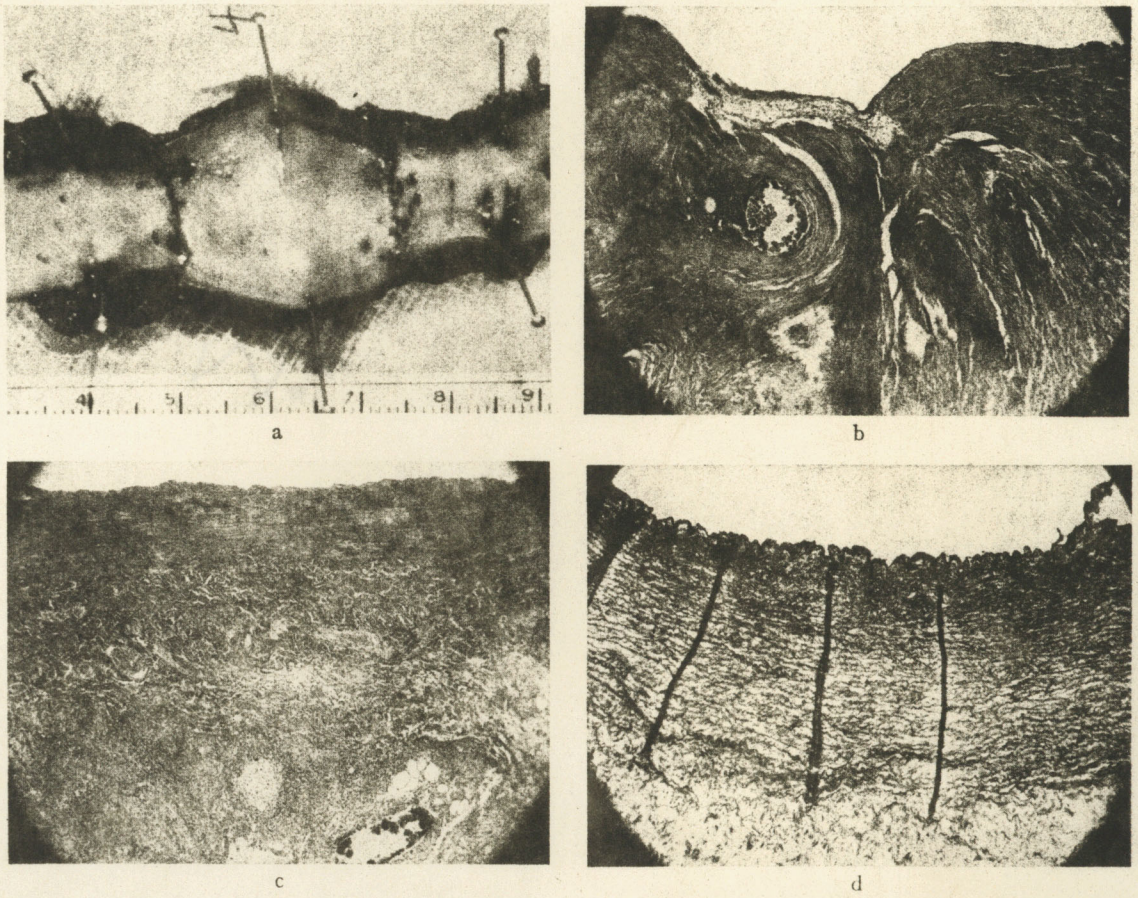


Fig. 1.[1] a, The graft stored 14 days has been in place for 3 days. b, At the suture line the tiny wedge-shaped thrombus has formed. c, The graft shows the absence of intima, essentially normal appearing media and acute inflammation in the adventitia. d, The elastic tissue appears to be normal.

failure of an arterial graft: (1) the viability of the vessel at the time of implantation; (2) the degree of immunological reaction between the host and the graft; and (3) the technique employed in making the anastomosis.

Insofar as the immediate (i.e. up to 1 year) *functional* result of arterial homografts in the dog is concerned, we believe the last named factor is the one of prime importance and the first two are of minor importance. The "viability" of the vessel will be discussed at some length in the following section of this report; the degree of immunological reaction which may be great in heterogenous transplants, has never appeared to have been of sufficient degree in our experience with homogenous transplants to jeopardize the survival of the graft. The immunological factors relating to nonautogenous grafts comprise a subject worthy of extensive investigation. However, as far as the *functional* success of the experimental homogenous arterial transplant is concerned, this has not appeared to be a decisive factor.

Technical aspects of the actual implantation of the vessel, on the other hand, appear to be of paramount importance. The well-established techniques of arterial anastomosis must, of course, be carefully observed. Rigid asepsis, delicacy in the handling of tissue,

[1]In all the figures in this paper, a is a photograph of the gross specimen, opened in the axis of the vessel, and viewed from the intimal side; b is a photomicrograph of the anastomosis (hematoxylin and eosin stain) with the graft always on the right and the host aorta on the left; c is a photomicrograph of the graft (hematoxylin and eosin stain); and d is the same section as c but with elastic tissue stain. All magnifications are × 45.

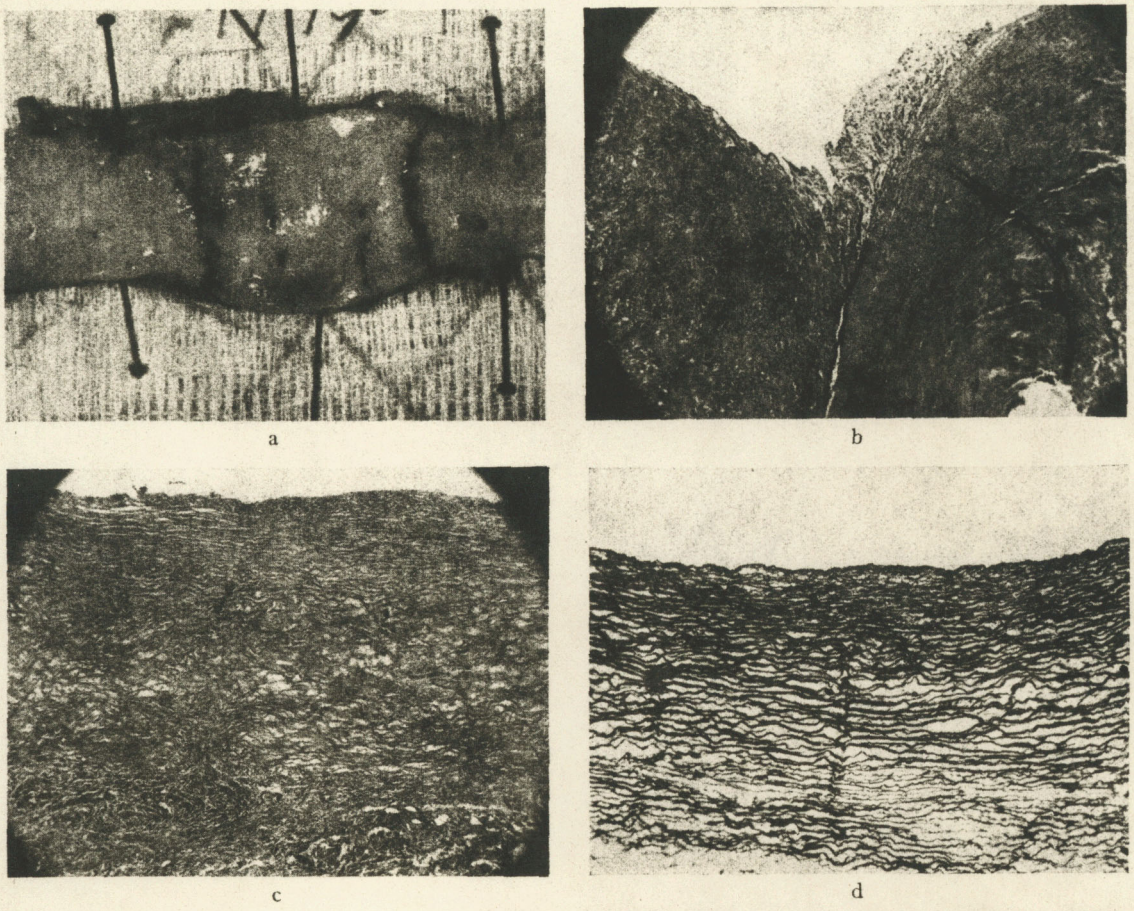


Fig. 2. a, The graft stored 21 days has been in place 14 days. b, The wedge-shaped thrombus shows invasion by fibroblasts and is beginning to extend down along the internal surface of the graft. c, Only a few of the smooth muscle cells of the media remain in this specimen. On the inner surface is a very thin plaque-like thrombus. The adventitia is now formed of granulation tissue. d, The elastic tissue appears normal.

avoidance of desiccation, the use of fine atraumatic needles with fine silk, the careful removal of the adventitia, and the avoidance of too large or too small a cuff are all matters of importance. We routinely used a series of continuous everting mattress sutures resulting in an intima-to-intima closure; other types of suture for effecting anastomosis may be equally effective (5).

The use of grafts, however, brings up other considerations than merely the technique of anastomosis. Of supreme importance in our experience is the size or diameter of the graft in relation to the diameter of the host vessel. The aorta when it is removed tends to contract somewhat. There does not appear to be further shrinkage during the time of storage. The capacity for distention remains as a function of the vessel, and upon being subjected to intraluminal pressure when implanted in the host will stretch to its previous in vivo diameter.

The creation of a vessel in continuity of uniform diameter under the hemodynamic conditions of blood flood is a law of vascular transplantation if uniformly successful results are to be expected. If the graft is allowed to exceed in diameter the host vessel, an aneurysm-like bulging occurs which results in turbulence and eddy currents in the flow of blood through the irregular channel. Intravascular thrombosis is favored, and clots may form usually

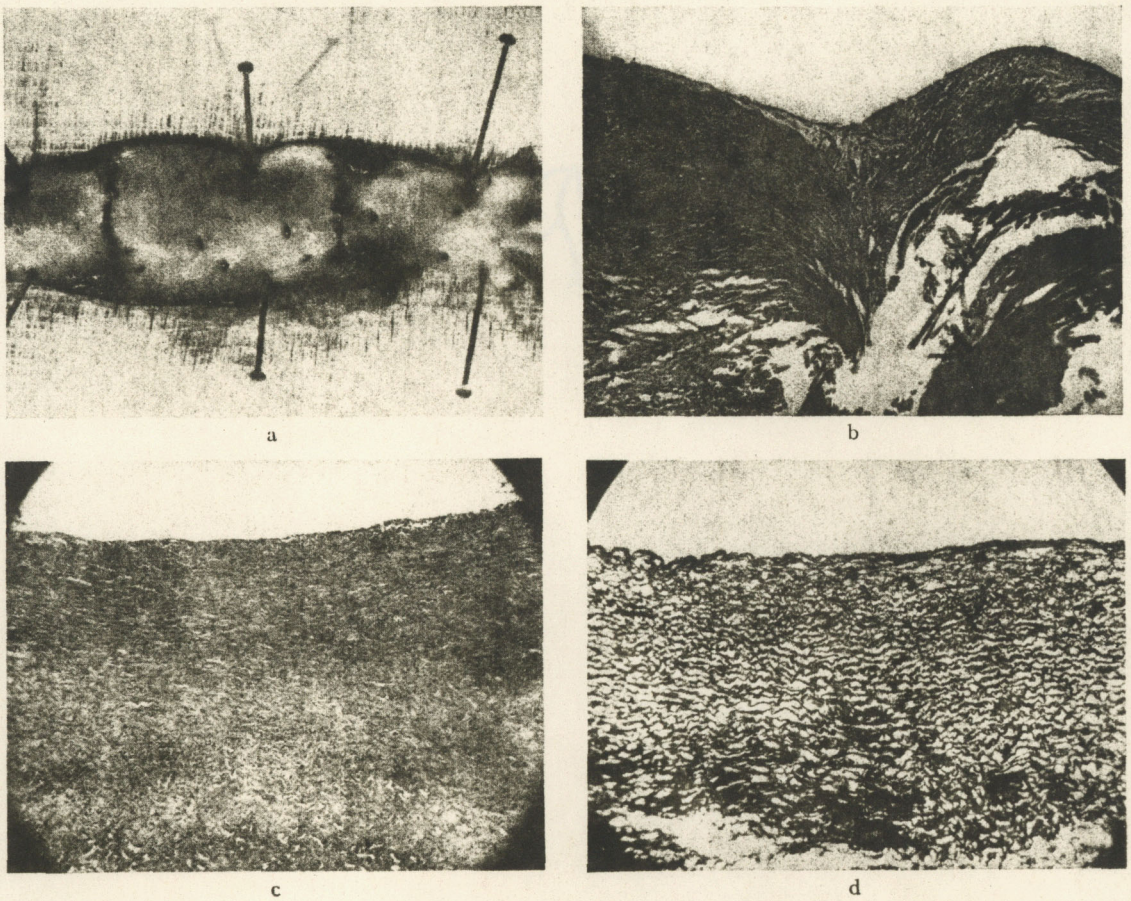


Fig. 3. a, The graft stored 10 days has been in place for 30 days. b, The wedge-shaped thrombus at the suture line has become further organized and has extended beyond the field down the internal side of the graft. Endothelial-like cells seem to line its surface. c, In this graft, smooth muscle survival in the media is very abundant. The adventitia is now composed of dense scar tissue. d, The elastic fiber pattern is relatively unchanged.

just distal to the proximal anastomosis gradually extending both distally and proximally until the lumen is obstructed and its value as a conduit ceases. Occasionally, degenerative changes in the graft occur under these conditions, with breakdown of tissue and secondary hemorrhage.

In choosing a suitable section of the stored aorta for implantation therefore, a segment should be selected which is slightly smaller in diameter than that of the normally pulsating host aorta. Under no circumstances is it wise to use a segment which is slightly or definitely larger than the host vessel. Care must then be taken to turn up as small a cuff as possible in order to avoid constriction at the suture line. In the early phase of our work the importance of this factor was not recognized, and no particular care was exercised in the choice of the graft for size, with the result that in the first few experiments thrombosis or hemorrhage supervened in over half of the animals. After realization of the phenomena involved, 36 aortic homografts have been performed with only 4 failures irrespective of the duration of storage of the graft.

EFFECT OF DURATION OF STORAGE UPON THE FUNCTIONAL SUCCESS OF THE HOMOGRAFT

Gross and his co-workers (2, 4) studied the effect of the duration of storage upon the "viability" of the blood vessel by tissue cul-

TABLE I.—EXPERIMENTAL AORTIC HOMOGRAFTS

Dog No.	Duration storage of graft days	Duration in host before sacrifice days	Results	Pictured in report
			Series A	
49-64	7	5	Successful graft; microscopic study	
48-270	8	240	Successful graft; microscopic study	Fig. 6
49-3	8	7	Successful graft; microscopic study	
49-109	8	45	Successful graft; microscopic study	
48-338	8	120	Successful graft; microscopic study	Fig. 5
48-351	10	30	Successful graft; microscopic study	Fig. 3
49-122	10	3	Died; rupture proximal suture line (graft too big)	
49-84	14	3	Successful graft; microscopic study	Fig. 1
48-344	15	2	Successful graft; died of intussusception	
49-77	17	1	Successful graft; microscopic study	
49-136	21	14	Successful graft; microscopic study	Fig. 2
49-89	22	90	Successful graft; (spec. lost down sink intact)	
49-19	29	21	Successful graft; microscopic study	
48-332	38	180	Successful graft; microscopic study	
48-219	40	60	Successful graft; microscopic study	Fig. 4
48-339	40	150	Successful graft; microscopic study	
			Series B	
49-143	45	30	Successful graft; microscopic study	
49-137	45	60	Successful graft; no photo obtained	
48-358	52	120	Successful graft; microscopic study	
48-357	56	150	Successful graft; microscopic study	
48-299	58		Successful graft, dog still living (transplanted January 19, 1949)	
49-21	59	7	Successful graft; died of intussusception; microscopic study	
49-150	59	60	Successful graft; microscopic study	
49-2	60	18	Successful graft; microscopic study	
49-4	64	180	Successful graft; microscopic study	Fig. 7
48-233	65		Successful graft; dog still living (transplanted January 26, 1949)	
49-15	65	220	Successful graft; microscopic study	
48-232	70		Successful graft; dog still living (transplanted January 18, 1949)	
48-110	70	90	Successful graft; microscopic study	
49-16	71	7	Died; rupture of suture line	
49-244	76	2	Died; rupture and thrombosis	
49-26	81		Successful graft; dog still living (transplanted February 11, 1949)	
49-23	84	4	Died; thrombosis, microscopic study	
49-57	121	42	Successful graft; animal killed in fight, microscopic study	
49-115	180	75	Successful graft; microscopic study	Fig. 8

ture methods. They found that "viability" as measured by fibroblastic proliferation in tissue culture media was enhanced by the storage of a large segment of vessel and by the use of an airtight stopper on the storage bottle. Under these conditions, vessels stored in balanced salt solution with 10 per cent serum showed a high percentage of fibroblast viability up to 37 days. Thereafter, the percentage of such viability appeared to decrease. Their series of ex-

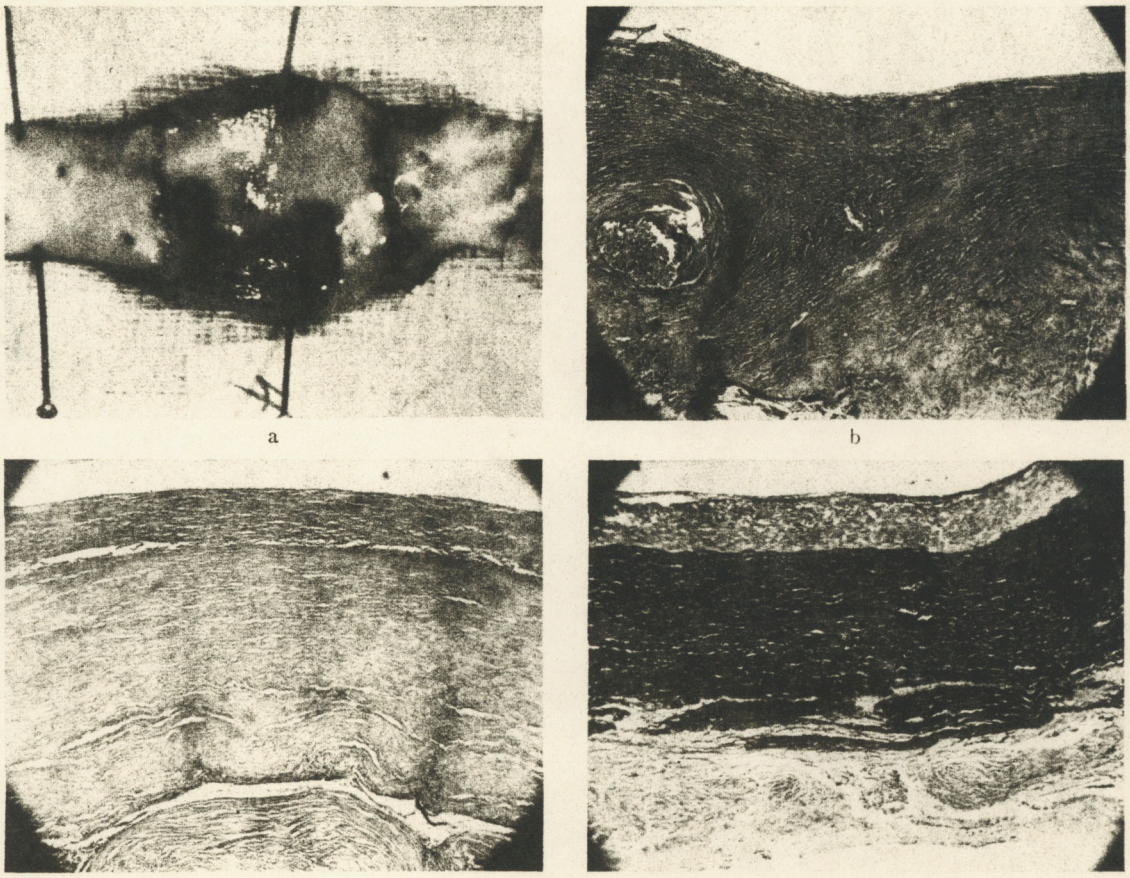


Fig. 4. a, The graft stored 40 days has been in place for 60 days. This graft shows more extensive surface thrombosis than is usual. b, At the suture line, the continued organization and differentiation of the wedge thrombus has resulted in a fibrocellular layer which smooths the contour of the lumen. c, The fibrocellular layer which grows in from the ends now covers the internal surface of the graft. In this specimen, no smooth muscle cells are seen in the media. The adventitia is becoming hyalinized. d, The elastic fibers appear very dense, possibly because with the loss of smooth muscle cells the total thickness of the media is diminished and the elastic fibers are more compact.

periments with storage of 50 days or more was quite small (3 observations) and it is possible that fibroblast viability may occasionally persist for even longer periods of time. However, in their experimental transplantations, 2 grafts stored longer than 50 days failed to function and became thrombosed. They conclude that a graft is more apt to be successful if it is "viable" when transplanted.

Our experience has led us to believe that the immediate success of the properly transplanted graft is not materially affected by the duration of storage, at least for periods up to 6 months. A series of aortic grafts stored from 40 to 180 days were implanted and allowed to remain in the host for various lengths of time before sacrificing the animal for histologic examination. Nineteen such transplantations were performed, with only 3 failures from thrombosis or hemorrhage. At the present time, a graft stored 70 days has been functioning for a period of 10 months in an animal apparently in perfect health. Table I is a summary of the pertinent data of our series of aortic homografts.

To say that the duration of storage has little apparent effect on the immediate success of the homogenous arterial transplant is not to say that it may not have a significant effect upon the ultimate outcome. Histologic evi-

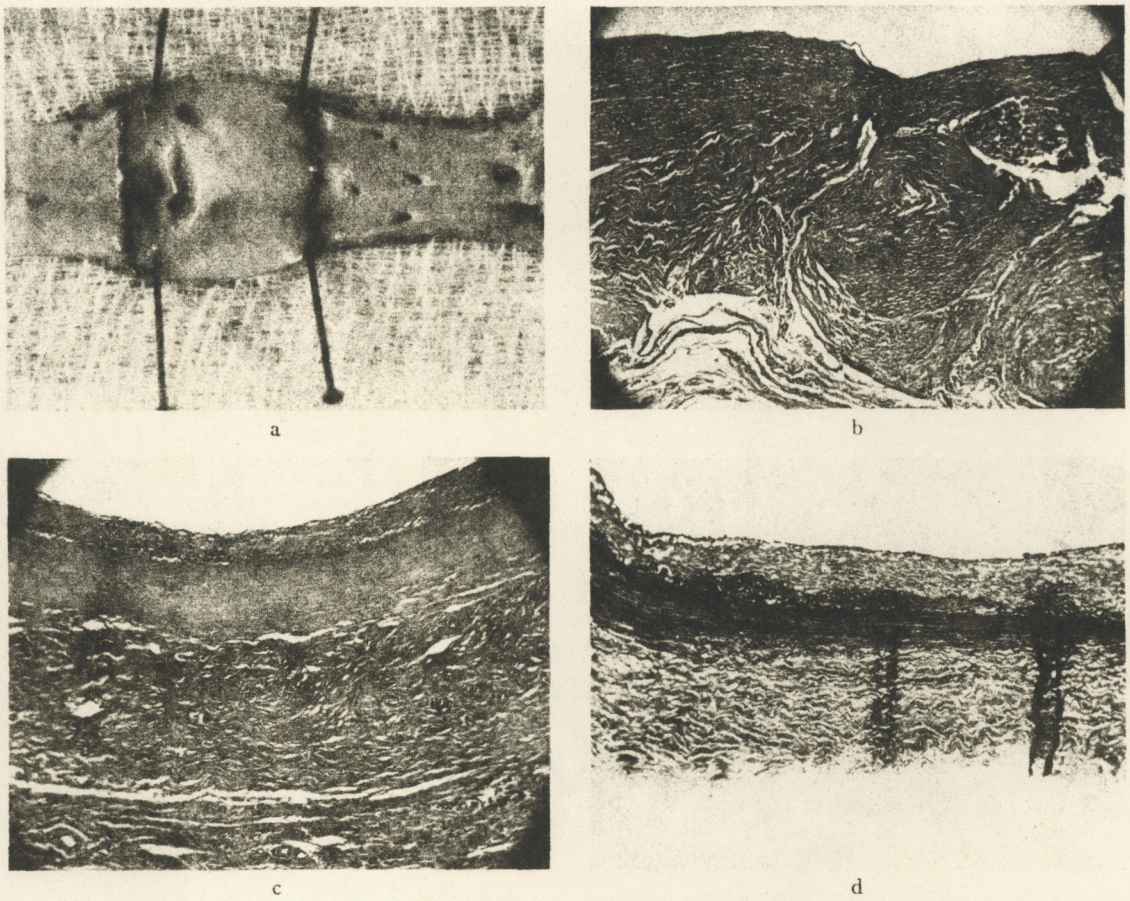


Fig. 5. a, The graft stored 8 days has been in place for 120 days. b, At the suture line the fibrocellular layer has smoothed the contour of the lumen. The surface layer of endothelium appears quite definite. Differentiation of the fibrocellular layer is apparent. c, The media is thin and compact. No cells are visible. The adventitia is a thick, dense, hyalinized scar. d, The elastic fibers appear less dense and the layer is thinner. It is apparent that new elastic fibers have been formed in the layer of fibrocellular tissue.

dence suggests that there are differences in the cellular structure of the resultant vessel between grafts stored for short periods of time and those stored for long periods. The "old" grafts demonstrate more pronounced degenerative changes earlier than those seen in "young" grafts. The nature of these changes is described in more detail in the following section of this paper, but we wish to emphasize that, although the immediate functional results using "old" grafts is equivalent to those obtained using "young" ones, our period of observation at this time (10 months) is too short to allow opinion as to the ultimate fate of these grafts, and there is reason to believe that "young" grafts (i.e. less than 40 days storage) may ultimately prove to be more durable.

HISTOLOGIC EVIDENCE ON THE FATE OF THE ARTERIAL HOMOGRAFT

In our experiments, we used the abdominal aorta just above the inferior mesenteric artery as the recipient area. The aorta was mobilized for a distance of about 4 centimeters, divided between special broad-bladed noncrushing clamps, and the ends allowed to contract. Into the gap a graft 2 centimeters in length was placed. This graft was carefully matched for size as described, and therefore might be taken

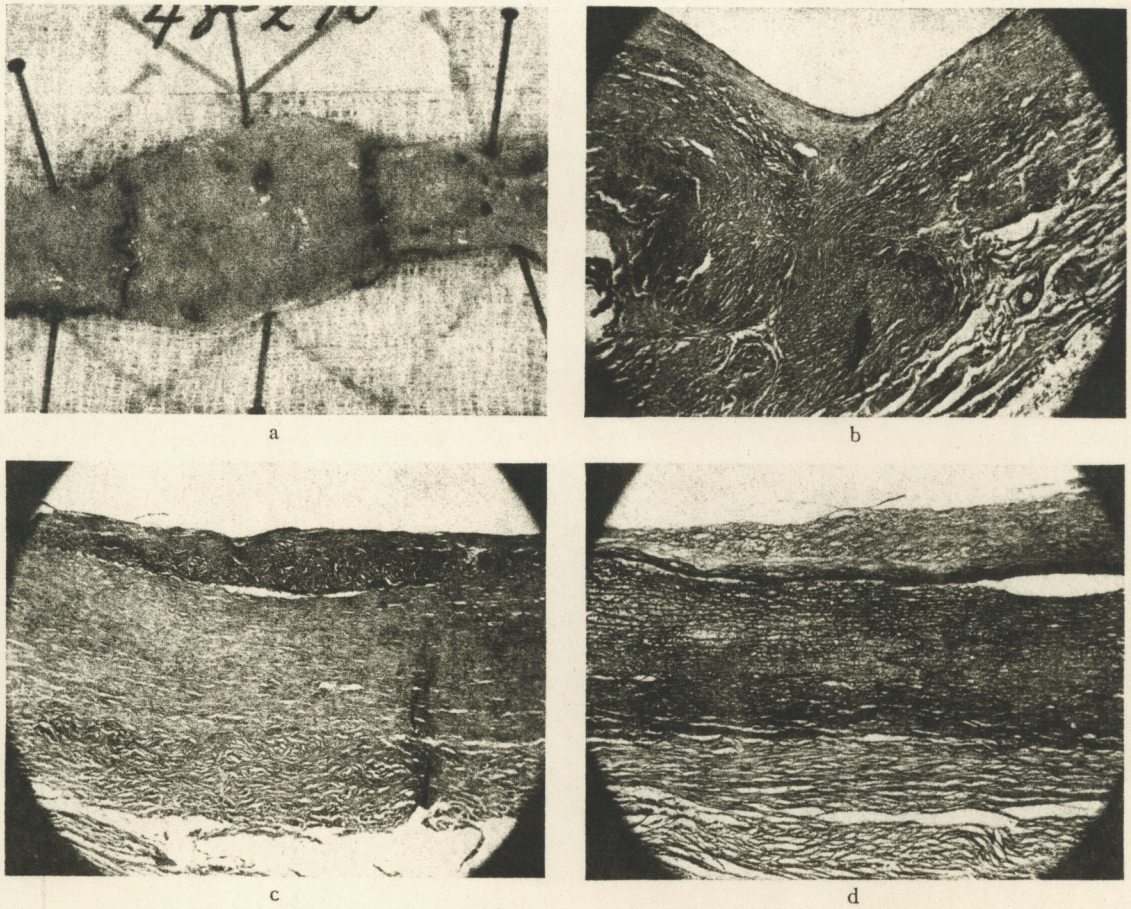


Fig. 6. a, The graft stored 8 days has been in place for 240 days. b, The anastomosis is firmly healed. c, The fibrocellular layer shows further differentiation and some degenerative changes. There is noticeable a spotty deposition of calcium throughout the layer and there are some smooth muscle cells visible in the media. d, The basal portion of the fibrocellular layer contains many elastic fibers. The elastic tissue of the media shows relatively normal architecture although the fibers are somewhat thin.

from the thoracic end of the donor aorta or the abdominal end, as the host vessel demanded. There are some minor histologic differences between the thoracic and the abdominal aorta in dogs relating chiefly to the thickness of the media and the abundance of elastic tissue, both of which are greater at the thoracic end. This factor introduced some minor quantitative variations in the resultant vessels but appeared to have no qualitative effect.

We have not thoroughly investigated what influence the length of the graft has on the ultimate survial. A few pilot experiments led us to believe that longer grafts survive and function equally as well as short ones and this was corroborated by a recent clinical experience in which one of us (H.S.) successfully resected a mycotic aneurysm of the thoracic aorta in a 17 year old boy and restored continuity of the vessel by the use of a stored graft 8 centimeters in length. The histologic data presented in this report, however, are all derived from transplants approximately 2 centimeters long.

Since the fate of the 3 layers of the aortic graft is apparently different, it seems convenient to describe them separately. The course of events is reconstructed from serial observations made on grafts which had been allowed to remain in the host from 1 to 240

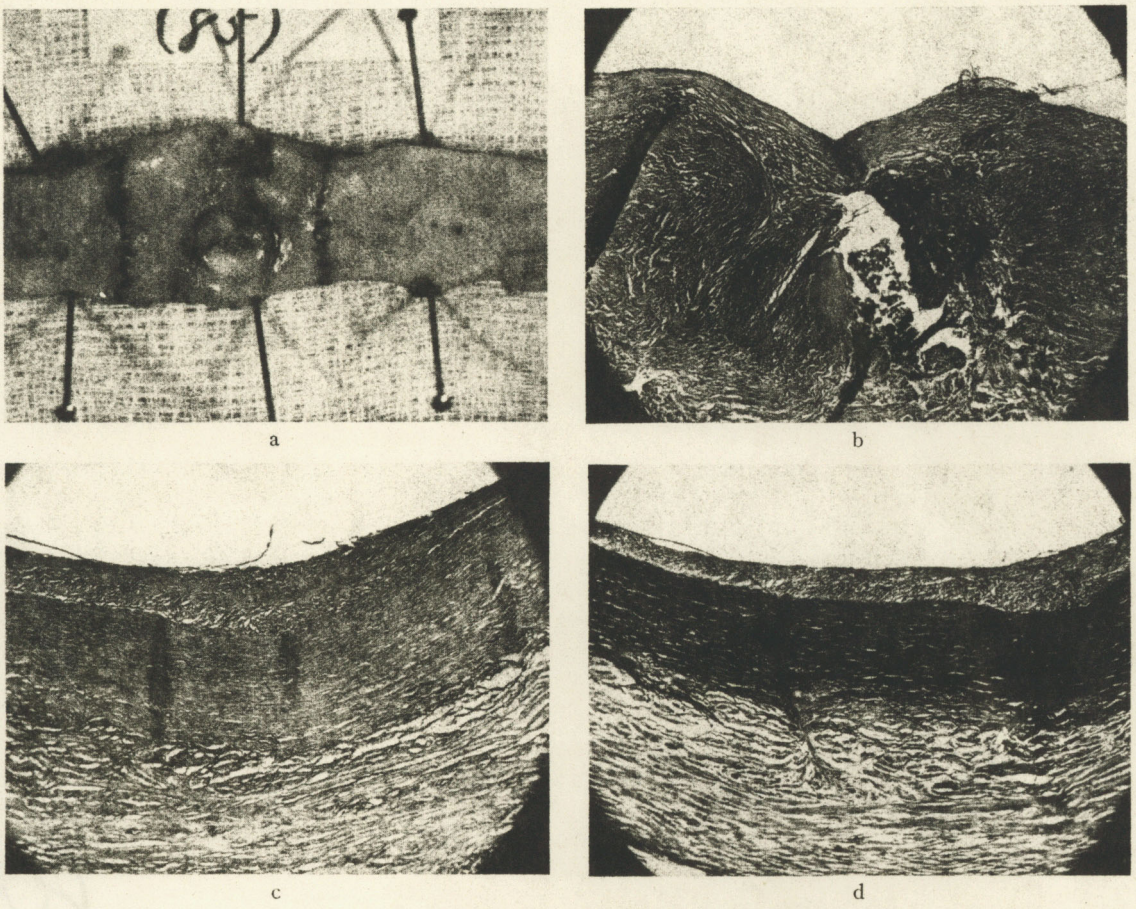


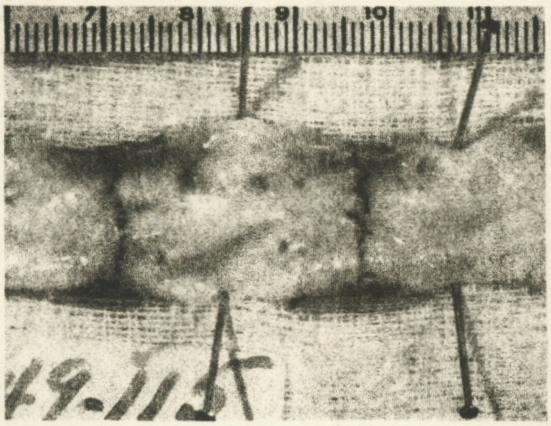
Fig. 7. a, The graft stored 64 days was in place for 180 days. A large calcium plaque is visible in the center of the graft. b, The suture line is similar to those seen with younger grafts. c, The fibrocellular layer is well differentiated and is sprinkled with calcium. No cells are visible in the media. d, The elastic tissue is dense and compressed.

days. The fate of "young" grafts stored from 8 to 40 days is described first.

Grafts stored less than 40 days. 1. The adventitia. Within 24 hours there is evidence of an acute inflammatory reaction on the part of the host evidenced by edema, fibrin deposition, extravasation of red blood cells, and infiltration of polymorphonuclear neutrophiles. This reaction reaches its height on about the third to fifth day. Degeneration of the donor fibroblasts in the adventitial layer is apparently extensive. Thereafter, healing begins by the infiltration of lymphocytes and capillary buds and fibroblasts. This granulation tissue gradually becomes more fibrous and eventually, by the end of the second month, quite collagenous. This whole process does not appear to differ in any significant way from the usual reponse to inflammation and degeneration, namely, healing by formation of scar tissue. Eosinophiles and plasma cells are not in evidence. This scar, for the first 3 months, may be much thicker than the original layer, but gradually thins out until by the sixth month it approximates the thickness of the original layer.

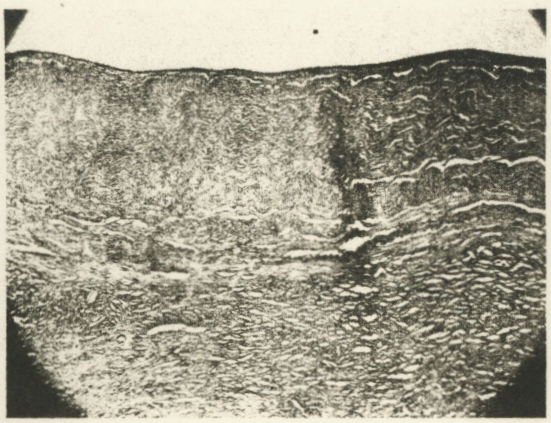
Thus, the adventitia of the donor vessel apparently degenerates and is entirely replaced by scar formation from the host. By the end of the second month, or perhaps earlier, it appears to be capable of contributing strength to the grafted segment.

2. The intima. At the end of 24 hours no visible trace of the intima of the donor vessel



a

b



c

d

Fig. 8. a, The graft stored 180 days was in place for 75 days. b, The anastomosis is well healed. The fibrocellular layer is thin in this section, yet in other areas, as seen in d, is much thicker. c, The media shows no survival of smooth muscle cells. There is diffuse and patchy deposition of calcium throughout the media of the stained section which does not show well in this black and white photomicrograph. d, The elastic tissue appears very dense and compressed. It apparently has remained almost totally intact.

remains. The luminal surface is apparently formed by the internal elastic membrane of the media. There is no evidence of inflammatory reaction; occasionally patchy areas of very thin thrombi occur on the internal elastic membrane. A tiny wedge-shaped ring thrombus appears in the angle where the graft was everted for anastomosis. About the seventh day a growth of fibroblasts appears in this tiny thrombus, cells apparently derived from the media of the host, although perhaps from the intima. From these cells a pannus-like growth progresses from each end of the graft centrally forming a fibrocellular layer which eventually, usually by the third month, covers the entire luminal surface. This layer is not perfectly uniform but varies in thickness from a single cell in some areas to many cells in others. It tends to obliterate the angle at the anastomosis and to smooth the luminal contour of the vessel at this point. It rarely grows onto the recipient aorta's intima for any significant distance but usually becomes fused to it at the arc of eversion. Thus, by the end of the second month, the vessel has acquired from the host a new intima, albeit somewhat thicker and irregular.

Subsequently this layer shows remarkable powers of differentiation. The surface cells tend to become flattened and resemble endo-

thelium. Immediately beneath this layer, the cells have a foamy appearance. Hyalin matrix is laid down and even new elastic fibrils are formed. By the fifth month spotty areas of calcification and some deposition of lipoids, presumably cholesterol, may be observed.

It appears, therefore, that the donor intima disappears entirely only to be replaced by a fibrocellular layer derived from the host by centripedal growth from the ends. This layer by the fifth month shows both differentiation and degeneration, and by this time may be contributing to the total strength of the grafted segment.

3. The media. There appears to be more variation in the fate of the media than in that of the 2 previously described layers, but these differences seem to be merely quantitative variations of a basic pattern. For a few days, the media looks entirely normal. About the fifth day the nuclei of the smooth muscle cells begin to disappear as if the cells were degenerating. Very little inflammatory reaction accompanies this process, which continues until about the end of the fourth week. Thereafter, no further loss of smooth muscle appears to take place. The quantitative extent of this loss varies from 100 per cent in some grafts to only an estimated 50 per cent in others. Certainly in some specimens, smooth muscle cells "take" in abundance and survive for at least 8 months. By the end of the third week a pink-staining hyalin material has appeared diffusely throughout the media. The greater the smooth muscle degeneration the greater the hyalinization. This is probably hyalin degeneration rather than the elaboration of a fibrous tissue matrix as no fibroblastic proliferation is apparent during this episode.

Meanwhile, the elastic tissue fibers survive intact and continue to show little tendency to degenerate or disappear. Because of the loss of smooth muscle cells, the total thickness of the media decreases and the elastic fibers are compressed closer together. In extreme examples the media, after 4 months, consists exclusively of densely packed elastic tissue embedded in a hyalin matrix. At the opposite extreme, where there has been good survival of smooth muscle, the elastic tissue is only moderately compressed, and the general architecture of the narrowed media appears almost normal. Plaque-like calcium deposits appear in the media by the fourth to sixth month in vessels in which smooth muscle degeneration has been extensive. In the presence of such calcium depositions, elastic fibers may become locally fragmented.

The healing between the media of the graft with that of the host at the site of anastomosis is accomplished by a thin layer of fibrous tissue. The ends remain everted, and there is no tendency for the two to flatten out and become a single layer in continuity. There is no fusion of elastic tissue between the donor and the recipient vessels.

Thus, in sharp contrast to the intima and adventitia which are replaced by the host, some or most of the media of the graft appears to survive and remains relatively intact for at least 8 months. The elastic tissue always survives and, in these "young" grafts, smooth muscle may persist in varying degrees. Calcification occurs in inverse proportion to smooth muscle survival. During the first 30 to 60 days, the strength of the graft must reside primarily in the elastic tissue of the media.

Grafts stored longer than 40 days. The chain of histological events in the adventitia and intima of transplants which have been stored longer than 40 days appears to be identical with that previously described for "younger" grafts. The media, however, differs in that there is no survival of smooth muscle cells. The smooth muscle degeneration seen in the second, third, and fourth weeks progresses to completion and the media is left as a thin layer of compact elastic tissue in a hyalin matrix. By the third or fourth month, calcium deposition is commonly seen in patchy distribution, and in these areas degeneration of elastic fibers may be evident.

It is this evidence of early significant degenerative changes in the media which should lead to caution in the use of grafts stored longer than 40 days in spite of their consistent effectiveness as conduits of blood in the months immediately following implantation. We have several living animals both with "young" and "old" grafts which we propose to follow over a period of years in an attempt to evaluate the outcome of these transplants.

SUMMARY

1. Canine arterial (aortic) homografts can be successfully stored in Ringer's solution containing 10 per cent homogenous serum in an ordinary electric refrigerator at 3 to 8 degrees C.

2. For consistent successful transplantation of aortic homografts, the proper matching in size of the donor and recipient vessels is a factor of paramount importance.

3. The duration of storage (up to 6 months) does not appear to be a significant factor governing the immediate functional success of the transplant. However, microscopic evidence suggests that degenerative changes appear earlier and are more extensive in grafts stored longer than 40 days than in those stored for a lesser period.

4. Serial microscopic studies indicate that the adventitia and the intima of the graft are totally replaced by similar layers derived from the host.

5. At least a part of the media of the graft apparently survives. The elastic tissue invariably remains for periods up to 10 months. Some smooth muscle cells in varying degree survive in grafts stored less than 40 days; no smooth muscle cells survive in grafts stored longer than 40 days.

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